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Working with Randy: The Diacylglycerol Acyltransferase Story

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Abstract Vegetable oils are one of the main agricultural commodities. Demand has been increasing steadily over the last five decades and, with finite land available, it is vital that we increase productivity. My laboratory has focused on the regulation of plant lipid metabolism and, as part of this work, we identified diacylglycerol acyltransferase (DGAT) as important at regulating carbon flux during oil accumulation. This led to collaborations with Randy Weselake's research group when we quantified the importance of DGAT in oilseed rape by using flux control analysis. Later, with David Taylor, we showed that over-expression of DGAT boosted oil accumulation in field-grown crops by around 8%. These studies led to a multitude of experiments with different oil crops, such as oil palm and soybean, as well as many rewarding collaborations with Randy.

Keywords Diacylglycerol acyltransferase · Kennedy pathway · Metabolic control analysis · Oil crops · Oilseed rape (*Brassica napus*) · Triacylglycerol synthesis

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Abbreviations

BUCA	bottom-up control analysis
DAF	days after flowering
DAG	diacylglycerol
DAP	days after pollination
DGAT	diacylglycerol acyltransferase
FW	fresh weight
GPAT	glycerol 3-phosphate acyltransferase
LPAAT	lysophosphatidic acid acyltransferase
MCA	metabolic control analysis
PAPase	phosphatidic acid phosphatase
TAG	triacylglycerol
TDCA	top-down control analysis

Introduction

Lipids have several major roles in organisms—as storage compounds (usually triacylglycerol [TAG]), as major components of membranes, as lipid-derived signaling molecules and as surface components (waxes, cutin and suberin in plants) (Gunstone et al., 2007; Gurr et al., 2016). My research has focused on the biosynthesis of acyl lipids and, in particular, on its regulation (Harwood, 2019). In the early nineties we turned our attention to oilseed rape (*Brassica napus*), one of the major oil crops. There are two distinct types of *B. napus*, the low erucate (LEAR) varieties (called Canola in Canada), utilized for food and the high erucate (HEAR) varieties, mainly used for industrial purposes (Gunstone et al., 2007). Canola varieties produce an oil which contains a high proportion of oleate (62%) and has a “healthy” ratio of n-6/n-3 polyunsaturated fatty acids (21% linoleate, 10% alpha-linolenate). Such oilseed rape is the third most important vegetable oil (15% total world production) (Weselake et al., 2017) and dominates oil

production in Northern Europe and Canada (Harwood et al., 2017).

In order to generate background data, we examined changes in the major acyl lipids of rapeseed embryos during development. This study showed the steady increase in TAG during the period of rapid development (20–40 days after pollination (DAP)) but, unexpectedly, there was also an increase in diacylglycerol (DAG) during this period (Table 1) (Perry and Harwood, 1993a). We interpreted this transient accumulation of DAG as indicating that the activity of diacylglycerol acyltransferase (DGAT) might limit TAG synthesis at times of high lipid accumulation (Perry and Harwood, 1993a). These observations were followed up by radiolabeling studies, which again indicated that DGAT could significantly control carbon flux and, indeed, was likely to be the main Kennedy pathway enzyme to exert significant flux control (Perry and Harwood, 1993b).

One of the pieces of evidence to indicate that DGAT could exhibit the most flux control within the Kennedy pathway (during rapid accumulation of seed oil) was that only DAG accumulated significantly during the radiolabeling experiments (Perry and Harwood, 1993b). We followed this observation up by using novel NMR techniques, together with our colleagues in Grenoble (Perry et al., 1999). The data confirmed that DAG accumulated to five-times the level of the next most abundant intermediate and, furthermore, that DGAT had the lowest activity of the four enzymes in the Kennedy pathway (Table 2) (Perry et al., 1999).

In our original analysis of developing oilseed rape embryos, there was a small decrease in TAG per embryo after 40 DAP which coincided with the dehydration of seeds (Perry and Harwood, 1993a). This decrease was later shown to be due to lipase activity and could represent losses of about 10% in the final oil yield (Kelly et al., 2013).

The above observations with oilseed rape led to two developments. First, the perceived importance of DGAT

Table 2 Kennedy pathway enzyme activity and intermediate levels in developing rapeseed embryos

	Total activity (nmol/min/gFW)	Substrate conc. (mg/gFW)
GPAT	10.0 ± 0.5	0.0387
LPAAT	95.0 ± 2.8	0.27 ± 0.09
PAPase	77.5 ± 5.9	0.81 ± 0.02
DGAT	3.8 ± 0.2	4.0 ± 0.1

Embryos were at 30 DAF and incubated in the light. Data as means ± SD (*n* = 3), taken from Perry et al. (1999).

was noted by Randy Weselake (then at the University of Lethbridge, Alberta) who had begun his important work on this enzyme. Second, we were encouraged to apply the technique of metabolic control analysis to oil accumulation in crops, having successfully applied it to lipid biosynthesis in leaves where light stimulation of lipid formation was shown to be due primarily to the activity of acetyl-CoA carboxylase (Page et al., 1994).

Metabolic Control Analysis

Metabolic control analysis (MCA) is a method for examining metabolic pathways and, in particular, of providing quantitative measures of constraints in the process—thus giving evidence for important control or regulatory points. Originally developed by the pioneering laboratories of Kacser and of Rapoport (Heinrich and Rapoport, 1974; Kacser and Burns, 1973) the techniques have been thoroughly discussed by Fell in a book (Fell, 1997).

The theory of MCA shows that the control of flux is distributed throughout a pathway. Thus, the idea (often promoted in textbooks) that there is a single “rate-limiting” step or enzyme reaction in a pathway is mis-guided. Furthermore, it is often ignored that, as conditions change, the control of metabolism along a pathway or between pathways will almost invariably alter also.

The first application of MCA to lipid biosynthesis was when we examined light-stimulated fatty acid (lipid) synthesis in the leaves of monocotyledons (barley, wheat) (Page et al., 1994). We used a version of MCA termed “Bottom-Up” Control Analysis (BUCA) where a particular reaction is targeted by use of a specific inhibitor or other means of altering it (*e.g.* by changing expression of an enzyme). An alternative method is termed Top-Down Control Analysis (TDCA) where the pathway is divided into parts connected by an intermediate. TDCA is useful because it provides an immediate overview of the whole pathway (Quant, 1993). Thus, it will provide information about the regulation exercised by large sections of the

Table 1 Fresh weight and non-polar lipids in developing rapeseed embryos

DAP	Values/embryo		
	Fresh wt. (mg)	DAG (mg)	TAG (mg)
17	0.81	2.2	27.4
21	0.97	4.6	85.6
25	2.85	10.1	252.7
31	3.62	16.9	400.1
41	4.53	28.2	970.8
46	4.03	19.1	954.0
Mature	2.80	11.9	935.5

Data taken from Perry and Harwood (1993a).

pathway. Furthermore, it does not depend on the ability to manipulate the activity of individual reactions specifically. For the use of TDCA applied to lipid formation in plants, the overall synthesis was divided into two blocks of reactions. Block A consisted of the *de novo* biosynthesis of fatty acids (in plastids) and Block B reactions were those of lipid assembly, mainly by the Kennedy pathway on the endoplasmic reticulum. The two Blocks were connected by the acyl-CoA intermediates (see Ramli et al., 2002b).

We first published on the use of TDCA in plants as part of a session organized by the Biochemical Society (Harwood et al., 1999) and, later, as part of my Society of Chemical Industry International Lecture Award. They were later referred to in a review article about olive and other fruits (Salas et al., 2000) and then, in more detail, with reference to oil palm and olive fruits (Ramli et al., 2002a, b). Further analysis of the accumulation of oil in fruits revealed important differences between oil palm and olive fruits (Ramli et al., 2005). Because of its importance as the premiere oil crop (Weselake et al., 2017), we produced a detailed analysis of MCA in oil palm (Ramli et al., 2009).

Working Together

As a result of our joint research interests, Randy Weselake and I started to talk about DGAT and the possibility of working together. I visited Lethbridge and we talked more! This was followed by a post-doctoral fellow in my laboratory, Mingguo Tang, spending a short time there. His work contributed to a nice paper where we used a DGAT-overexpressing line of *B. napus* to see how transgenic manipulation could be used to boost oil yields.

In *B. napus*, as in many other plants, there is more than one DGAT isoform. DGAT-1 seems to be the main isoform concerned with the usual pattern of acyl-TAG molecular species while DGAT-2 appears to be prominent in those oil crops that accumulate unusual TAG (Cahoon et al., 2007). Previous work in Randy's laboratory had shown that DGAT-1 was the main isoform in *B. napus* embryos and we thought it would be the most effective at enhancing oil accumulation. In particular, we thought that it might be particularly important when oilseed rape was unlikely to reach its full potential due to environmental stress (Weselake et al., 2008). Overexpression of DGAT-1 increased DGAT activity and, as expected, reduced the DAG/TAG ratio in seeds. These data were consistent with DGAT being important for flux control (Perry et al., 1999; Perry and Harwood, 1993a, b). It was reassuring that the overexpression of DGAT in *B. napus* also gave increased oil yields for oilseed rape plants not only in the greenhouse but also in the field (Weselake et al., 2008) (Table 3). The seed

Table 3 Seed properties of *Brassica napus* overexpressed with *BnDGAT1* cDNA

	Westar	DI-2.20
DGAT Activity (umol/mn/mg, protein)	18.3 ± 0.8	78.5 ± 7.6
DAG/TAG ratio	0.09	0.05
Oil yield (%)—greenhouse growth	41.5 ± 1.9	47.7 ± 0.3
Oil Yield (%)—field grown	45.4 ± 0.4	47.5 ± 1.5

Data taken from Weselake et al. (2008) with means ± SD (*n* = 3). Activity and the DAG/TAG ratio were measured at 27DAF. The field conditions were for the summer of 2005 in Alberta.

oil content of transgenic lines increased between 5% and 12% when grown in fields under drought conditions in Saskatchewan in the summer of 2003. This was important because it reduced the losses due to drought in a number of lines. By the theory of MCA, overexpression of DGAT which exerted strong flux control should have changed the values for Group Flux Control Coefficients. Thus, in TDCA for *B. napus*, most control was in the lipid assembly portion of the overall pathway for oil accumulation (Ramli et al., 2002a, b). This changed in the overexpressing lines from 69% to 51% (Table 4) Weselake et al., 2008) thus showing that constraints in oil synthesis were lowered by overexpressing DGAT. Such observations demonstrated clearly the value of determining flux control values before going to the trouble of altering gene expression.

In a follow-up, Randy and I collaborated with David Taylor at the National Research Council of Canada (Saskatoon) to assess more fully the performance of DGAT-overexpressing lines in the field. We monitored growth and oil yield in two successive seasons (2006, 2007), one of which was drought stressed. In both years, there was a significant increase in oil yield—5–8% in 2006 and 3–7% in 2007 on a dry weight basis (Taylor et al., 2009). In the greenhouse, similar lines gave 10–16% increases in oil yield. These large-scale trials fully validated our proposals, first, that DGAT activity could be an important constraint on TAG biosynthesis and, second, that its overexpression could have a significant impact on commercial yields of *B. napus*.

Our joint work on the overexpression of DGAT in oilseed rape was also reported in several conference communications (e.g. Guschina et al., 2007; Weselake et al., 2007a, b) while the full details of MCA in *B. napus* were described in (Tang et al., 2012). It is noticeable that nowadays when efforts are made to increase oil yields in crops, increased DGAT expression is a favored tactic (e.g. Lardizabal et al., 2008; Vanherke et al., 2012). Indeed, Randy and I had already discussed the utility of DGAT overexpression for increasing oil yields in two review articles (Weselake et al., 2009a, b).

Table 4 Changes in the flux control characteristics on over-expressing DGAT in oilseed rape

	Group flux control coefficient	
	Fatty acid synthesis	Lipid assembly
Westar ($n = 5$)	0.31 ± 0.02	0.69 ± 0.02
D1-2.20 ($n = 3$)	0.49 ± 0.05	0.51 ± 0.05

Data taken from Weselake et al. (2008).

Around this time, the collaboration between Randy and myself took on a new dimension. The AOCS Lipid Library was looking for a new editor and I was encouraged to apply. While being interviewed and then offered the post, I became aware that Randy was also interested. So why not cooperate? Our appointment as joint editors-in-chief proved to be a happy time for me. Randy was conscientious, punctual, and always forthcoming with ideas. After a few years and with our retirements approaching, we handed the task to Alejandro Marangoni in 2017. We also made a few individual contributions (*e.g.* Harwood, 2010) and, I hope, left the site in good condition.

Subsequent to the main days of our collaborations, Randy and I have continued to author conference proceedings (Guschina et al., 2011), reviews (Chen et al., 2015; Harwood et al., 2013; Woodfield et al., 2015) and chapters in books (Harwood et al., 2017; Weselake et al., 2017). Since our initial work on DGAT, I have continued research with oilseed rape. This has involved some new collaborations which have revealed the subtleties of acyl lipid metabolism during oil accumulation (Woodfield et al., 2017) as well as the use of lipidomics to address the perennial question of how important are the relative contributions of DGAT and phospholipid: diacylglycerol acyltransferase (PDAT) for TAG formation in different plants (Woodfield et al., 2018). In particular, we have been interested in whether any other enzymes in the Kennedy pathway, apart from DGAT, could contribute significantly to the carbon flux into TAG formation. When we looked at lysophosphatidic acid acyltransferase (LPAAT), we found that its overexpression could raise TAG accumulation significantly and our collaborator, David Fell, was able to derive some new (and potentially very useful) equations to allow calculation of flux without using inhibitors to modify the radiolabeling of intermediates (Woodfield et al., 2019). Randy spotted our new publication and e-mailed me, even before he had received his special “New Phytologist” copy that we had recommended! He pointed out that raising LPAAT activity might increase levels of the phosphatidic acid intermediate. If this occurred then there was the possibility that DGAT would be stimulated (Caldo et al., 2018) and that subsequently could explain (part of?) the increase in oil yields in LPAAT overexpressors. Inspired by his

suggestion, I went back to the laboratory data we had and, at least in the radioisotope experiments, the levels of phosphatidic acid labeling were increased in our transgenic lines!

Perhaps this is an appropriate place to end this short article? Working with Randy has always been a pleasure. He is not only a fine scientist but also a gentleman. Our collaborations have, I believe, contributed positively to lipid science. Moreover, from the above example it seems that his impact is far from finished!

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Conflict of Interest The authors declare that they have no conflict of interest.

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